

## **Complement in Lupus Nephritis**

The complement cascade consists of a series of plasma proteins that not only play a vital role in destroying pathogens but also mediate humoral and cellular interactions within the immune system (Figure).

Recent work, driven particularly by the availability of gene-targeted mice, has considerably increased our understanding of the links between the complement system and glomerular disease. In this presentation I shall discuss recent insights into the role of the complement system in the pathogenesis of lupus nephritis.

Complement activation products typically deposit in glomeruli in systemic lupus erythematosus (SLE), where they are thought to contribute to injury but, paradoxically, deficiencies of early components of the classical pathway in man are amongst the strongest predisposing factors to lupus<sup>1</sup>. In addition low copy number of the gene for C4 also predisposes to SLE<sup>2</sup>. It has previously been shown that the classical pathway component C1q and mannose binding lectin (MBL) bind to apoptotic cell surface blebs, which contain high concentrations of lupus autoantigens. Deficiency of C1q leads to autoimmunity associated with impaired clearance of apoptotic cells by phagocytes and the appearance of glomerular apoptotic bodies<sup>3:4</sup>. These data led to the formulation of the 'waste disposal' hypothesis which proposes that dying cells provide the source of autoantigens responsible for driving autoantibody production in SLE and that defects in the clearance mechanisms for these dying cells increases the risk of developing autoimmunity<sup>1</sup>. It is increasingly recognised that classical pathway activation by C1q and subsequent C3 deposition on the surface of apoptotic cells occurs in a predominantly IgM-dependent manner<sup>5</sup> This is consistent with the finding that serum IgM deficient mice develop autoimmunity<sup>6</sup>

Dendritic cells (DC) play a central role in the control of immune responses. Immature DC (iDC) are able to induce tolerance whereas DC, which have matured in response to inflammatory signals, are stimulatory. Importantly, therefore, opsonisation of apoptotic cells with C1q (or MBL) enhances the uptake of apoptotic material not only by macrophages but also by iDC<sup>7</sup> In addition, iDC are a rich source of C1q the production of which is down-regulated on iDC maturation<sup>8</sup>. Thus, secretion of cytokines such as interferon alpha, which lead to maturation of dendritic cells and

reduced secretion of C1q, may potentially thereby impair clearance of apoptotic cells and thus predispose to inflammation and autoimmunity. C1q may also regulate the threshold for DC activation

Autoantibodies that bind to the collagenous tail of C1q are well described in SLE<sup>9</sup>. There is a strong correlation between anti-C1q antibodies and renal disease in SLE; titres of anti-C1q antibodies may predict lupus nephritis flares and they can be eluted from kidney biopsies in lupus nephritis<sup>10</sup>. A series of papers by Daha's group have elucidated the role of these autoantibodies in lupus nephritis. His group demonstrated that lupus prone mice develop circulating anti-C1q antibodies that deposit in the kidney before the development of overt nephritis<sup>11</sup>. This suggested that anti-C1q antibodies were present in the right place and at the right time to be involved in the pathogenesis of glomerular injury but did not provide proof of their pathogenicity. They then injected rabbit-anti-mouse C1q antibodies into a non-autoimmune strain of mouse. This caused C1q and anti-C1q and in addition C3 to deposit in glomeruli but only caused mild albuminuria<sup>12</sup>. Most recently this same group synthesised mouse anti-mouse C1q antibodies which when injected into non-autoimmune strains of mice, depleted circulating C1q levels and led to the deposition of C1q and IgG within glomeruli but again caused only minor renal injury<sup>13</sup>. The same antibodies were then administered to Rag2<sup>-/-</sup> (immunoglobulin deficient) mice. This led to a reduction in circulating C1q but no glomerular C1q deposition implying that IgG in the glomerulus acts as a target for the attachment of C1q which can then bind anti-C1q antibodies. Most importantly, it was demonstrated that if anti-C1q antibodies were given together with complement-fixing antibodies directed against glomerular basement membrane, marked C1q and immunoglobulin deposition occurred together with significant glomerular inflammation that did not occur if either antibody was administered alone. This study provides the definitive evidence that anti-C1q antibodies can exacerbate antibody-mediated glomerular injury. The JL-1 antibody used by the authors recognises the same collagen-like domain as do human anti-C1q antibodies suggesting that this murine study is likely to be relevant to human SLE. Using gene targeted mice it was shown that injury in this model was dependent on C3, C4 and Fc receptors. The authors hypothesised that activation of the classical pathway by anti-C1q antibodies led to generation of chemotactic complement fragments, inflammatory cell influx and stimulation of these cells via Fc receptors. An alternative mechanism

for the action of anti-C1q may be related to C1q depletion which may lead to autoimmunity as described earlier.

More information on the role of the lectin pathway in the pathogenesis of lupus is also starting to appear. MBL variant alleles associated with lower functional levels of MBL are common and may predispose to SLE and in particular to nephritis. In patients with SLE these variant alleles are associated with anti-C1q and antiphospholipid antibodies but not with anti-MBL antibodies<sup>14</sup>. Anti-MBL antibodies are however present in a number of patients with idiopathic SLE but unlike anti-C1q antibodies do not correlate with nephritis or disease activity<sup>15</sup>. MBL has been shown to bind predominantly to late apoptotic cell blebs, and thereby to activate complement in a similar fashion to C1q<sup>16</sup>. The resultant C4 deposition is able to enhance the non-inflammatory phagocytosis of apoptotic cells by macrophages and immature dendritic cells which may help maintain tolerance<sup>7</sup>.

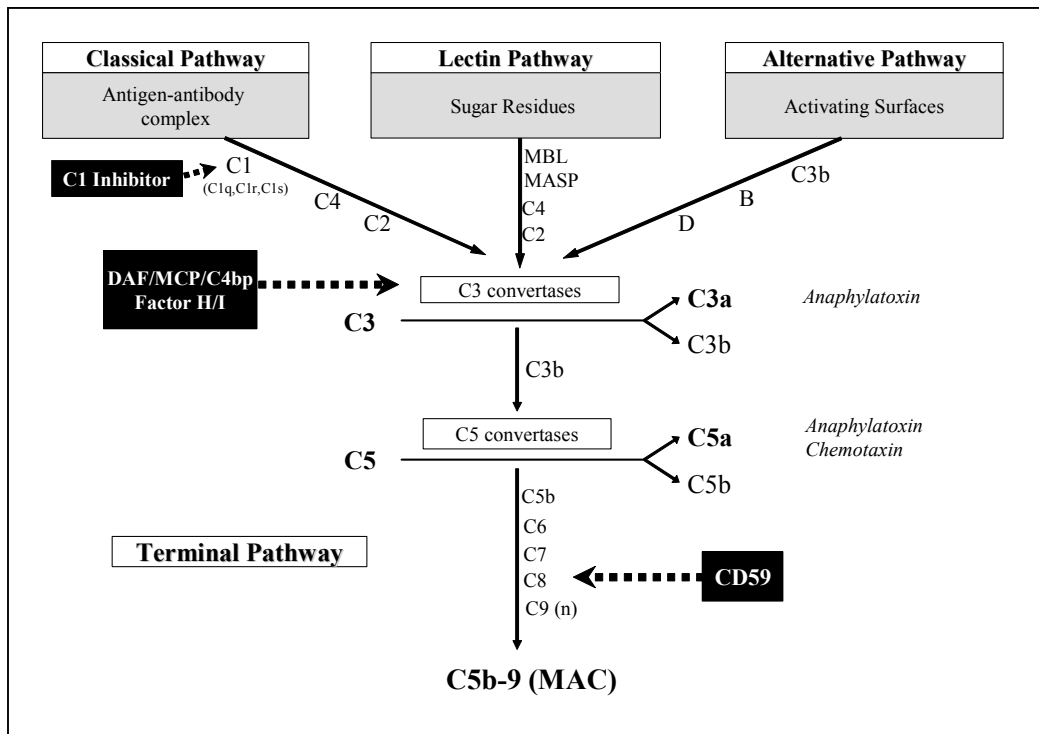
In contrast to the role of the classical pathway in protecting from the development of autoimmunity, activation of the alternative pathway may contribute to tissue damage via the formation of the anaphylatoxins or the membrane attack complex. It was previously shown that lupus-prone (MRL/lpr) mice deficient in the alternative pathway protein Factor B were protected from renal disease compared to wild-type controls<sup>17</sup>. However, this study was flawed as there were important differences in MHC haplotype between the mice which may have accounted for some of the phenotypic variability. In order to clarify this, the same investigators have studied the effect of deficiency of another component of the alternative pathway, Factor D<sup>18</sup>. In this study they confirmed a role for alternative pathway activation in the pathogenesis of nephritis in SLE. Factor D deficiency had no effect on serum IgG levels or glomerular IgG deposition, but significantly reduced glomerular hypercellularity, reduced glomerular C3 deposition and improved renal function. This is in contrast to mice deficient in C3 which were not protected from renal injury and in fact developed more proteinuria and greater glomerular IgG deposition than controls<sup>19</sup>. These results highlight the important differences between the protective effects of the classical pathway and the damaging effects of the alternative pathway in the pathogenesis of lupus nephritis. However, in spite of the improvement in renal injury, the lifespan of factor D deficient mice was not increased indicating the importance of factors other

than complement, such as Fc receptor-mediated processes, in causing glomerular injury in lupus<sup>20</sup>.

In order to examine the effect of the terminal pathway of complement activation on the pathogenesis of lupus nephritis Ravirajan et al<sup>21</sup> utilised a murine model of SLE induced by human monoclonal anti-dsDNA antibodies that is characterised by proteinuria and glomerulonephritis. They demonstrated that the administration of neutralising antibodies to C5 significantly reduced proteinuria and treated animals had less mesangial expansion and podocyte foot process effacement. This confirms previous findings that anti-C5 antibodies reduce renal disease in another murine lupus model (NZB/W)<sup>22</sup>. These studies suggest important therapeutic strategies for human lupus nephritis using agents that inhibit the terminal pathway whilst leaving the potentially beneficial effects of the classical pathway and C3 unaffected.

In summary there is evidence that elements of the classical pathway of complement activation protect against the development of SLE. Animal models suggest that mechanisms involved include clearance of apoptotic cells and other cellular debris, alteration of the activation threshold of dendritic cells and clearance of immune complexes. Complement activation occurs in glomeruli in lupus nephritis. Animal models indicate an injurious role for the alternative and terminal pathways of complement activation and also for anti-C1q antibodies in amplifying glomerular inflammation.

Figure. The complement system can be activated by the classical, mannose-binding lectin or alternative pathways. In each case this results in the formation of a C3 convertase enzyme which activates C3 and culminates in the synthesis of the anaphylatoxins C3a and C5a, the opsonin C3b, and the membrane attack complex (MAC). The complement system is very tightly regulated at the levels of C1, the C3 or C5 convertases and within the terminal pathway by both membrane bound and circulating factors (marked in black).



#### Reference List

1. Manderson AP, Botto M, Walport MJ: The role of complement in the development of systemic lupus erythematosus. *Annu.Rev.Immunol.* 22:431-456, 2004
2. Yang Y, Chung EK, Wu YL, Savelli SL, Nagaraja HN, Zhou B, Hebert M, Jones KN, Shu Y, Kitzmiller K, Blanchong CA, McBride KL, Higgins GC, Rennebohm RM, Rice RR, Hackshaw KV, Roubey RA, Grossman JM, Tsao BP, Birmingham DJ, Rovin BH, Hebert LA, Yu CY: Gene copy-number variation and associated polymorphisms of complement component C4 in human systemic lupus erythematosus (SLE): low copy number is a risk factor

for and high copy number is a protective factor against SLE susceptibility in European Americans. *Am.J.Hum.Genet.* 80:1037-1054, 2007

3. Mitchell DA, Taylor PR, Cook HT, Moss J, Bygrave AE, Walport MJ, Botto M: C1q protects against development of glomerulonephritis independently of C3 activation. *J.Immunol.* 162:5672-5679, 1999
4. Botto M, Dell'Agnola C, Bygrave A, Thompson EM, Cook HT, Petry F, Loos M, Pandolfi PP, Walport MJ: Homozygous C1q deficiency causes glomerulonephritis associated with multiple apoptotic bodies. *Nature Genetics* 19:56-59, 1998
5. Quartier P, Potter PK, Ehrenstein MR, Walport MJ, Botto M: Predominant role of IgM-dependent activation of the classical pathway in the clearance of dying cells by murine bone marrow-derived macrophages in vitro. *Eur.J.Immunol.* 35:252-260, 2005
6. Ehrenstein MR, Cook HT, Neuberger MS: Deficiency in serum immunoglobulin (Ig)M predisposes to development of IgG autoantibodies. *J.Exp.Med.* 191:1253-1257, 2000
7. Nauta AJ, Castellano G, Xu W, Woltman AM, Borrias MC, Daha MR, van Kooten C, Roos A: Opsonization with C1q and mannose-binding lectin targets apoptotic cells to dendritic cells. *J.Immunol.* 173:3044-3050, 2004
8. Castellano G, Woltman AM, Nauta AJ, Roos A, Trouw LA, Seelen MA, Schena FP, Daha MR, van Kooten C: Maturation of dendritic cells abrogates C1q production in vivo and in vitro. *Blood* 103:3813-3820, 2004
9. Uwatoko S, Mannik M: Low-molecular weight C1q-binding immunoglobulin G in patients with systemic lupus erythematosus consists of autoantibodies to the collagen-like region of C1q. *J.Clin.Invest* 82:816-824, 1988
10. Holers VM: Anti-C1q autoantibodies amplify pathogenic complement activation in systemic lupus erythematosus. *J.Clin.Invest* 114:616-619, 2004
11. Trouw LA, Seelen MA, Visseren R, Duijs JM, Benediktsson H, de Heer E, Roos A, van Kooten C, Daha MR: Anti-C1q autoantibodies in murine lupus nephritis. *Clin.Exp.Immunol.* 135:41-48, 2004
12. Trouw LA, Duijs JM, van Kooten C, Daha MR: Immune deposition of C1q and anti-C1q antibodies in the kidney is dependent on the presence of glomerular IgG. *Mol.Immunol.* 40:595-602, 2003
13. Trouw LA, Groeneveld TW, Seelen MA, Duijs JM, Bajema IM, Prins FA, Kishore U, Salant DJ, Verbeek JS, van Kooten C, Daha MR: Anti-C1q autoantibodies deposit in glomeruli but are only pathogenic in combination with glomerular C1q-containing immune complexes. *J.Clin.Invest* 114:679-688, 2004
14. Seelen MA, van der Bijl EA, Trouw LA, Zuiverloon TC, Munoz JR, Fallaux-van den Houten FC, Schlagwein N, Daha MR, Huizinga TW, Roos A: A role for

mannose-binding lectin dysfunction in generation of autoantibodies in systemic lupus erythematosus. *Rheumatology.(Oxford)* 44:111-119, 2005

15. Seelen MA, Trouw LA, van der Hoorn JW, Fallaux-van den Houten FC, Huizinga TW, Daha MR, Roos A: Autoantibodies against mannose-binding lectin in systemic lupus erythematosus. *Clin.Exp.Immunol.* 134:335-343, 2003
16. Nauta AJ, Raaschou-Jensen N, Roos A, Daha MR, Madsen HO, Borrias-Essers MC, Ryder LP, Koch C, Garred P: Mannose-binding lectin engagement with late apoptotic and necrotic cells. *Eur.J.Immunol.* 33:2853-2863, 2003
17. Watanabe H, Garnier G, Circolo A, Wetsel RA, Ruiz P, Holers VM, Boackle SA, Colten HR, Gilkeson GS: Modulation of renal disease in MRL/lpr mice genetically deficient in the alternative complement pathway factor B. *J.Immunol.* 164:786-794, 2000
18. Elliott MK, Jarmi T, Ruiz P, Xu Y, Holers VM, Gilkeson GS: Effects of complement factor D deficiency on the renal disease of MRL/lpr mice. *Kidney Int.* 65:129-138, 2004
19. Sekine H, Reilly CM, Molana ID, Garnier G, Circolo A, Ruiz P, Holers VM, Boackle SA, Gilkeson GS: Complement component C3 is not required for full expression of immune complex glomerulonephritis in MRL/lpr mice. *J.Immunol.* 166:6444-6451, 2001
20. Clynes R, Dumitru C, Ravetch JV: Uncoupling of immune complex formation and kidney damage in autoimmune glomerulonephritis. *Science* 279:1052-1054, 1998
21. Ravirajan CT, Wang Y, Matis LA, Papadaki L, Griffiths MH, Latchman DS, Isenberg DA: Effect of neutralizing antibodies to IL-10 and C5 on the renal damage caused by a pathogenic human anti-dsDNA antibody. *Rheumatology.(Oxford)* 43:442-447, 2004
22. Wang Y, Hu Q, Madri JA, Rollins SA, Chodera A, Matis LA: Amelioration of lupus-like autoimmune disease in NZB/WF1 mice after treatment with a blocking monoclonal antibody specific for complement component C5. *Proc.Natl.Acad.Sci.U.S.A* 93:8563-8568, 1996

# **Complement in Membranous Nephropathy**

## **David J. Salant – Boston University Medical Center**

### **Membranous Nephropathy**

- Autoimmune disease
- Common cause of nephrotic syndrome in adults
- Idiopathic
- Secondary
  - Lupus nephritis – Class V
  - Hepatitis B-associated
  - Drugs
  - Cancer-associated

### **Pathological Features**

- Light microscopy
  - Normal → diffuse GBM thickening (spikes)
- Immunofluorescence
  - Granular capillary IgG and complement
- Electron microscopy
  - Subepithelial electron-dense deposits
  - Podocyte foot process effacement, cytoskeletal condensation and apical microvillous transformation

### **Pathogenesis**

- In-situ immune complex formation
- Circulating antibodies
- Target antigen on podocytes
  - Rats – megalin
  - Neonatal MN – alloimmune response to neutral endopeptidase (NEP)
  - Idiopathic and secondary MN – unknown

### **Experimental Membranous Nephropathy**

- Susceptible rat strains immunized with tubular brush border (Fx1A) develop proteinuria after 6-8 weeks and an immune complex GN indistinguishable from human MN
- Rats injected with anti-Fx1A develop passive HN and proteinuria within 5 days
- Subepithelial immune deposits form in situ when circulating antibody binds to an intrinsic glomerular antigen

### **Role of Complement in Experimental MN**

- Passive Heymann nephritis (PHN) in rats
  - Complement depletion prevents proteinuria
  - Proteinuria and sublethal podocyte injury require formation of C5b-9
  - Depletion of C6 prevents proteinuria
  - Urinary excretion of C5b-9 corresponds to complement-mediated proteinuria
- Active Heymann nephritis
  - Only rats with complement deposits become proteinuric
  - Proteinuria depends on neutralization of Crry (C-regulatory protein)
- “Planted” subepithelial antigen
  - C6-deficient rabbits protected

### **Evidence for a Role for Complement in Human Membranous Nephropathy**

- C3 and C5b-9 are detected in most cases of recent onset MN
- C3d (stable product of C3) is present in all cases of MN
- C5b-9 is detectable in the urine of cases of recent onset MN
- Development of proteinuria in neonatal anti-NEP-induced MN is dependent on the deposition of C-fixing IgG1 antibodies

### **Caveats**

The PVG strain of C6-deficient rats develop proteinuria after induction of PHN  
The predominant antibodies in human MN are non-C-fixing IgG4

### **Effects of C5b-9 in Cultured Glomerular Epithelial Cells**

- Increased intracellular calcium (influx and release from stores)
- Activation of
  - Protein kinases – RTKs, PKC, ERK, JNK, p38
  - Phospholipases – PLC, cPLA<sub>2</sub>
  - Transcription factors – NFκB
  - Enzymes – Cox-2, NADPH oxidase, MMP-9, heparanase
  - Stress pathways – ER stress, Hsp27
  - Growth factors – PDGF-B, HB-EGF, TGF-β
  - CDK inhibitors – p21, p27
- Matrix protein production – type IV collagen, laminin, heparan sulfate proteoglycans
- DNA damage
- Disruption of the actin cytoskeleton and cell-matrix adhesion complexes

## **Effect of Complement on the Podocyte Slit Diaphragm in Experimental Membranous Nephropathy**

- Nephritin, a major podocyte slit-diaphragm protein, is linked to the actin cytoskeleton by CD2AP and anchored in the plasma membrane by podocin
- Slit-diaphragms are dislocated at the onset of proteinuria in PHN
- Complement-dependent podocyte injury in PHN causes nephritin to dissociate from actin, which may explain the dislocation of slit-diaphragms and account for the onset of proteinuria
- The amount of nephritin is reduced, in part due to loss in the urine

## **Treatment of Idiopathic Membranous Nephropathy**

### Standard Treatment

- ACEi or ARB, diuretic, statin
- High-risk patients:
  - Pulse steroid alternating with cytotoxic (Ponticelli)
  - Oral cyclophosphamide
  - Cyclosporin
  - Mycophenolate mofetil?

### Targeted Treatment

- Anti-CD20 (Rituximab)
- Humanized anti-C5 monoclonal antibody (eculizumab)

## **Treatment of Idiopathic Membranous Nephropathy with Complement Inhibitor anti-C5 monoclonal antibody (eculizumab)**

A multi-center double-blind controlled study of eculizumab was performed in 130 patients with idiopathic MN. While there was no effect on the primary endpoint of proteinuria after 16 weeks of therapy, some patients given up to one-year of this agent in an open-label extension had impressive responses (unpublished).

## **Treatment of Idiopathic Membranous Nephropathy with Rituximab (anti-CD20)**

- Two small case series induced a complete or partial remission of proteinuria in 50-60% of patients.
- There was no way of predicting which patients would respond

## **Future Directions**

- Identify the membranous nephropathy antigen
- Develop a sensitive and specific immunoassay for anti-MN antibodies
- Screen MN patients for circulating MN antibodies
- Select only those with high titers of anti-MN antibodies (active disease) for therapy with complement inhibitors or anti-CD20

### Selected references

1. **Cybulsky AV, Quigg RJ, and Salant DJ.** Experimental membranous nephropathy redux. *Am J Physiol Renal Physiol* 289: F660-671, 2005.
2. **Debiec H, Nauta J, Coulet F, van der Burg M, Guignonis V, Schurmans T, de Heer E, Soubrier F, Janssen F, and Ronco P.** Role of truncating mutations in MME gene in fetomaternal alloimmunisation and antenatal glomerulopathies. *Lancet* 364: 1252-1259, 2004.
3. **Kon SP, Coupes B, Short CD, Solomon LR, Raftery MJ, Mallick NP, and Brenchley PE.** Urinary C5b-9 excretion and clinical course in idiopathic human membranous nephropathy. *Kidney Int* 48: 1953-1958, 1995.
4. **Quigg RJ.** Role of complement and complement regulatory proteins in glomerulonephritis. *Springer Semin Immunopathol* 24: 395-410, 2003.
5. **Schulze MS, Donadio JV, Pruchno CJ, Baker P, J., Johnson RJ, Stahl RAK, Watkins S, Martin DC, Wurzner R, Gotze O, and Couser WG.** Elevated urinary excretion of the C5b-9 complex in membranous nephropathy. *Kidney Int* 40: 533-538, 1991.
6. **Shankland SJ.** Cell-cycle control and renal disease. *Kidney Int* 52: 294-308, 1997.
7. **Spicer ST, Tran GT, Killingsworth MC, Carter N, Power DA, Paizis K, Boyd R, Hodgkinson SJ, and Hall BM.** Induction of passive Heymann nephritis in complement component 6-deficient PVG rats. *J Immunol* 179: 172-178, 2007.
8. **Yuan H, Takeuchi E, Taylor GA, McLaughlin M, Brown D, and Salant DJ.** Nephritin dissociates from actin, and its expression is reduced in early experimental membranous nephropathy. *J Am Soc Nephrol* 13: 946-956, 2002.

## Complement in ANCA Vasculitis and Glomerulonephritis

J.Charles Jennette, M.D.

University of North Carolina at Chapel Hill

Antineutrophil cytoplasmic autoantibodies (ANCA) are associated with the most common form of aggressive glomerulonephritis in adults, pauci-immune necrotizing and crescentic glomerulonephritis (PICGN) (1). Approximately three quarters of patients with PICGN have systemic small vessel vasculitis, including microscopic polyangiitis and Wegener's granulomatosis (1). There is now compelling *in vitro*, experimental animal, and clinical evidence that ANCA IgG causes pauci-immune necrotizing and crescentic glomerulonephritis and systemic small vessel vasculitis (2-4).

By definition, PICGN has a paucity of immunoglobulin deposited in glomeruli, as well as a paucity of complement components (1). This suggests that the pathogenic mechanism of ANCA-associated disease is different from that of typical immune complex disease. In line with this concept, complement, which is clearly involved in many immune complex diseases, was not obviously incriminated in the pathogenesis of pauci-immune ANCA-associated disease. Unexpectedly, however, recent experimental animal experiments as well as *in vitro* experiments using human ANCA strongly implicate the alternative pathway of complement activation in the mediation of vascular inflammation by ANCA IgG (5,6).

Experimental animal studies that implicate the alternative complement system have utilized mouse models of glomerulonephritis induced by anti-myeloperoxidase (anti-MPO) antibodies (mimicking human MPO-ANCA) (3,4). Anti-MPO antibodies and lymphocytes are produced by immunizing MPO knockout mice with purified mouse MPO (3). Transfer of nephritogenic doses of anti-MPO B-lymphocytes into immune deficient mice, or transfer of nephritogenic doses of anti-MPO B-lymphocytes or purified anti-IgG into wild type mice causes NCGN and systemic small vessel vasculitis that is dependent of neutrophil influx and activation (3,4).

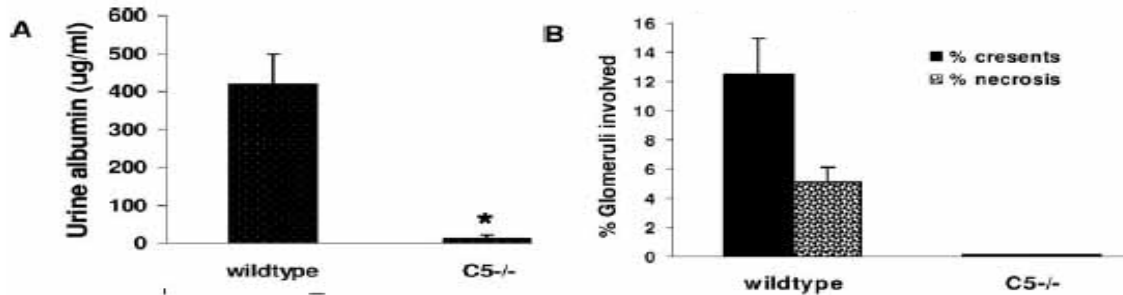
In one study (5), all wild type (WT) mice that receive a nephritogenic dose of anti-MPO IgG developed pauci-immune glomerulonephritis with crescents; and all immune deficient mice (Rag2<sup>-/-</sup>) that received nephritogenic doses of anti-MPO splenocytes developed circulating anti-MPO antibodies and resultant glomerulonephritis with crescents. However, this induction of disease was prevented by complement depletion using cobra venom factor (CVF) (Table 1), although circulating levels of anti-MPO were not affected. Complement depletion also abrogated influx of neutrophils and macrophages.

Table 1 (from reference 5)

Groups	Mice	% Mice with crescents/necrosis	Crescents (%)	Necrosis (%)	PMN (Gr-1)	Macrophage (CD68)
Anti-MPO IgG	WT	100	11.3 ± 1.2	5.3 ± 1.2	0.48 ± 0.17	0.82 ± 0.21
Anti-MPO IgG + CVF	WT	0	0.0 ± 0.0	0.0 ± 0.0	0.07 ± 0.03	0.10 ± 0.04
Anti-MPO splenocytes	Rag2 <sup>-/-</sup>	100	36.5 ± 21.5	35.8 ± 15.4	2.39 ± 0.47	1.76 ± 0.19
Anti-MPO splenocytes + CVF	Rag2 <sup>-/-</sup>	0	0.0 ± 0.0	0.0 ± 0.0	0.21 ± 0.07	0.11 ± 0.06

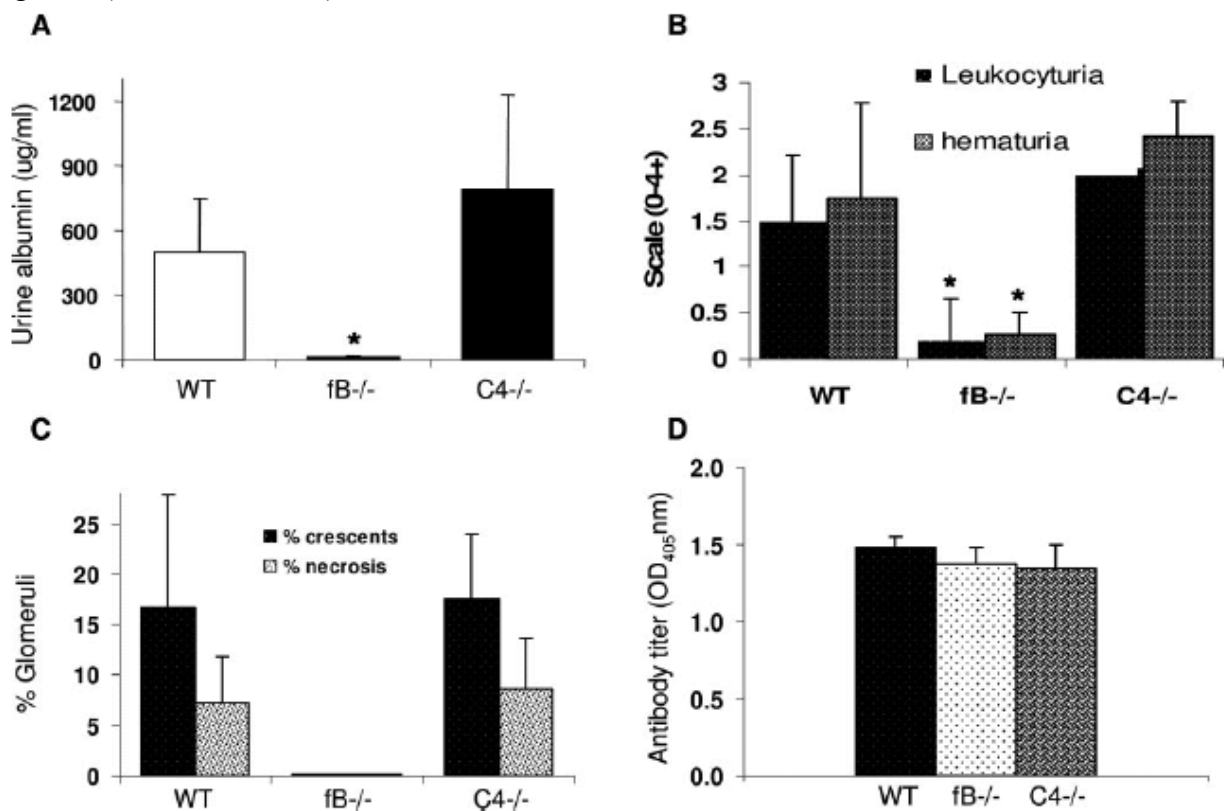
To confirm the effect of total complement blockade, mice deficient in the common complement pathway factor C5 (C5<sup>-/-</sup>) were injected with anti-MPO and compared to control wild type mice. All wild type mice developed glomerulonephritis with albuminuria and an average of 12% of glomeruli with crescents, whereas, C5<sup>-/-</sup> mice developed no proteinuria and no pathologic evidence for glomerulonephritis (Figure 1).

Figure 1 (from reference 5)



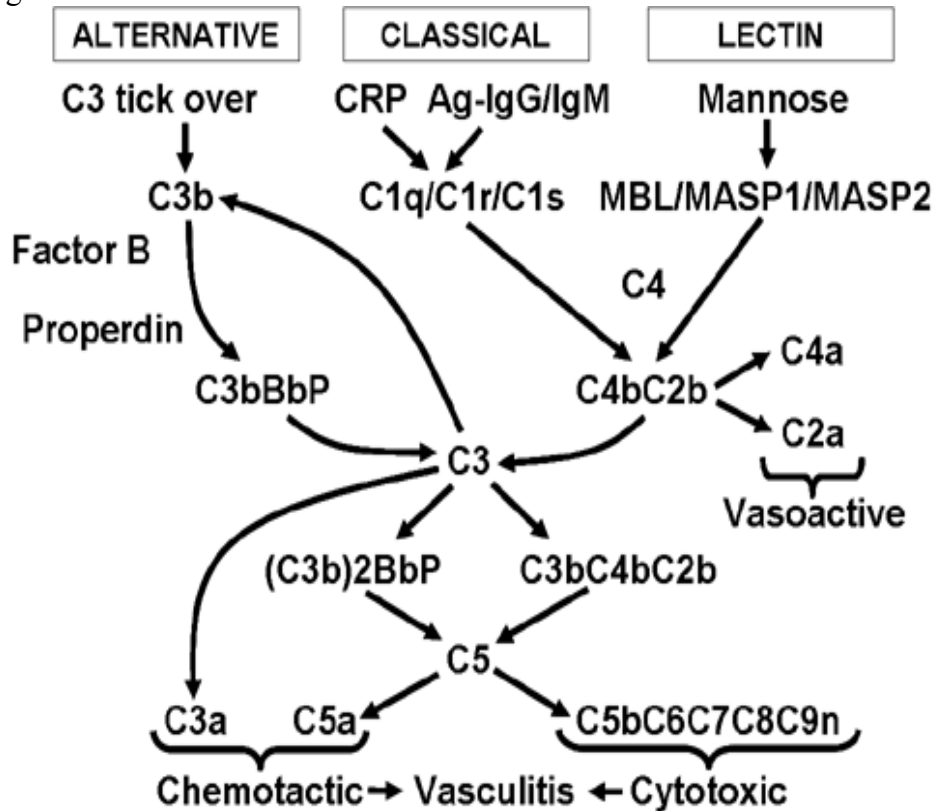
To identify which complement activation pathway was involved, mice with knockout of factor B (blocking the alternative pathway) or C4 (blocking the lectin and classical pathways) were compared to wild type control mice. As demonstrated in Figure 2, C4<sup>-/-</sup> mice had comparable disease to wild type, whereas Fb<sup>-/-</sup> mice were completely protected; indicating that the alternative pathway but not the classical or lectin pathways are involved in pathogenesis.

Figure 2 (from reference 5)



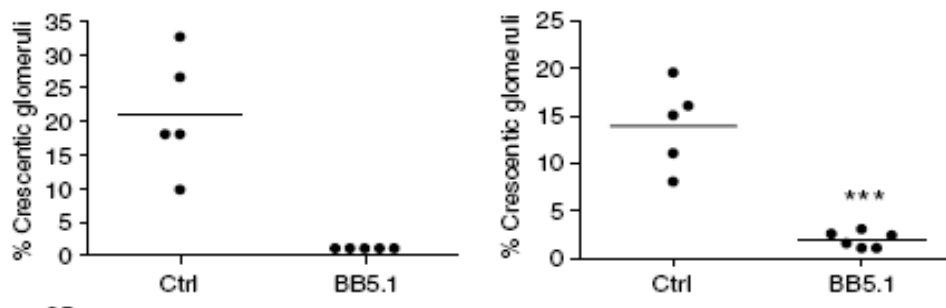
As illustrated in Figure 3, the alternative complement activation pathway has an amplification loop that generates a C3 convertase (C3bBbP) and C5 convertase ((C3b)2BbP) that generate the membrane attack complex as well as C5a, which is one of the most potent neutrophil attractants and activators known. Based on what is known about the pathophysiology of ANCA disease, including the pivotal role of neutrophil activation, the generation of C5a could be an important pathogenic event.

Figure 3:



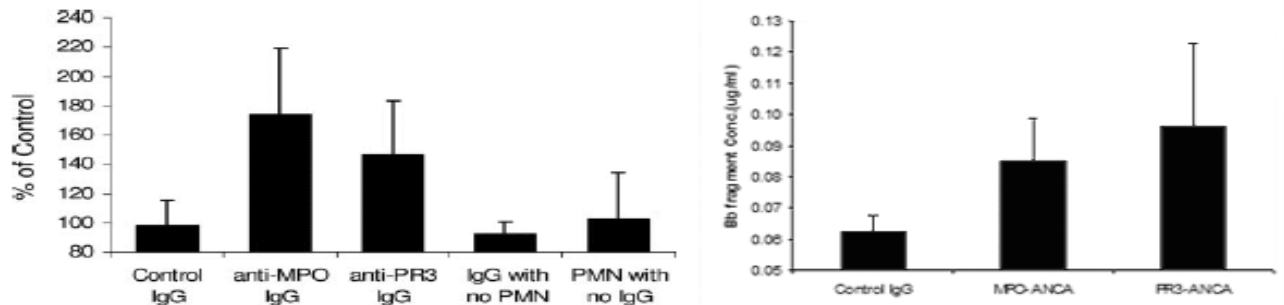
Based on the hypothetical importance of C5 and C5a, the effect of the C5 inhibiting monoclonal antibody BB5.1 was evaluated in murine glomerulonephritis induced by anti-MPO IgG (6). As shown in Figure 4, pretreatment with BB5.1 completely blocked development of glomerulonephritis (left panel) and administration of BB5.1 one day after disease induction substantially suppressed the severity of disease (right panel).

Figure 4 (from reference 6)



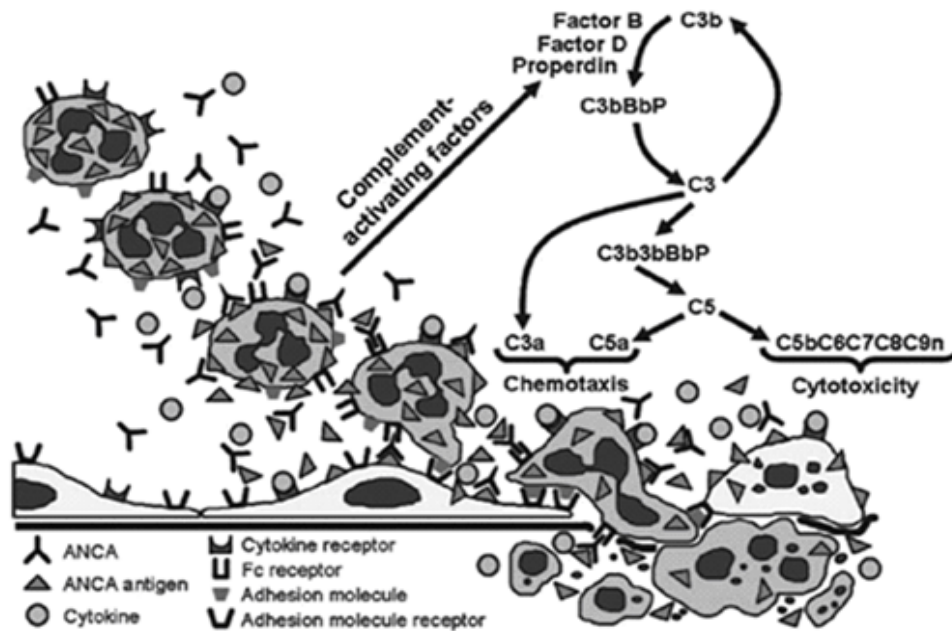
The studies in this mouse model of disease unequivocally support a major role for the alternative complement activation pathway in the induction of this model of disease, but they do not prove that complement is involved in human ANCA-associated disease. Thus, *in vitro* studies were designed not only to find evidence that complement could be involved in human disease but also to probe the mechanism of complement involvement. Human ANCA IgG specific for MPO (MPO-ANCA) and proteinase 3 (PR3-ANCA) was isolated and incubated with normal human neutrophils. The supernatant from this reaction was then reacted with normal plasma and any activation of complement measured. Both MPO-ANCA IgG and PR3-ANCA IgG, but not normal control IgG, were able to activate complement as monitored by generation of C3a (5) and Factor Bb (unpublished data) (Figure 5)

Figure 5 (from reference 5 on left, and unpublished data on right)



These observations support the hypothetical pathogenic process diagrammed in Figure 6, i.e., activation of neutrophils by ANCA IgG generates factor(s) that activate the alternative complement pathway, which amplifies the inflammation, probably through generation of C5a.

Figure 6 (from reference 5):



## References:

1. Jennette JC, Thomas DB: Pauci-immune and Antineutrophil Cytoplasmic Autoantibody Glomerulonephritis and Vasculitis in Heptinstall's Pathology of the Kidney, 6th Edition, Jennette JC, Olson JL, Schwartz MM, Silva FG (eds), Lippincott Williams & Wilkins, Philadelphia, 2007, chapter 14, 643-674
2. Jennette JC, Xiao H, Falk RJ. The pathogenesis of vascular inflammation by antineutrophil cytoplasmic antibodies. *J Am Soc Nephrol* 2006; 17:12356-1242
3. Xiao H, Heeringa P, Hu P, Liu Z, Zhao M, Aratani Y, Maeda N, Falk RJ, Jennette JC. Antineutrophil cytoplasmic autoantibodies specific for myeloperoxidase cause glomerulonephritis and vasculitis in mice. *J Clin Invest* 2002; 110:955-963.
4. Xiao H, Heeringa P, Liu Z, Huugen D, Hu P, Falk RJ, Jennette JC: A major role for neutrophils in anti-myeloperoxidase antibody induced necrotizing and crescentic glomerulonephritis. *Am J Pathol* 2005; 167:39-45
5. Xiao H, Schreiber A, Heeringa P, Falk RJ, Jennette JC: Alternative complement pathway in the pathogenesis of disease mediated by antineutrophil cytoplasmic autoantibodies. *Am J Pathol* 2007; 170:52-64.
6. Huugen D, van Esch A, Xiao H, Peutz-Kootstra CJ, Buurman WA, Cohen Tervaert JW, Jennette JC, Peter Heeringa P. Inhibition of complement factor C5 protects against anti-myeloperoxidase antibody-mediated glomerulonephritis in mice. *Kidney Int* 2007; 71:646-654

## *Complement in Renal Transplant Rejection*

*Robert B. Colvin, M.D.*

**Department of Pathology  
Massachusetts General Hospital  
Harvard Medical School  
Boston, MA 02114  
colvin@helix.mgh.harvard.edu**

The complement system plays multiple pathogenetic roles in rejection of renal allografts and one of the components, C4d, is an important diagnostic marker of antibody mediated rejection (1, 2). Complement proteins (e.g., C3) arose as an ancient, innate defense system in invertebrates that preceded the adaptive immune system in phylogeny. It is not surprising that during evolution these complement proteins and their receptors were incorporated into both the antibody and T cell mediated responses. C4 first evolved in conjunction with immunoglobulin. The Belgian microbiologist Jules Bordet (Nobel Prize 1919) discovered and defined complement as the factor(s) in fresh normal serum necessary for lysis of antibody coated red cells. The present discussion focusses on four topics: Ischemia/reperfusion injury, T cell mediated rejection, antibody mediated rejection and accommodation.

### **I. Ischemia/Reperfusion**

All renal transplants begin their sojourn in a new host after suffering ischemia and reperfusion injury (IRI). This is manifested by delayed graft function (DGF), defined as post-transplant requirement for dialysis, which occurs in 15-20% of deceased donor grafts and >90% of asystolic donor grafts (3). DGF is an adverse complication primarily due to the associated increased risk of acute rejection (4). The link between ischemia and increased immunogenicity may involve the complement system.

The complement system is an important mediator of IRI, as shown in the protection from IRI in mice that are genetically deficient in any of several complement components (2). In mouse renal models of ischemia, the alternative pathway is most important. Mice deficient in C3-, C5-, or C6 are protected from renal IRI, whereas C4-deficient mice were not (5). The lack of requirement for C4 does not exclude the MBL pathway, since MBL can bypass C4 (6). Tubular epithelial cells, rather than vessels, are the main site of injury. Antibody to C5a had little effect. Thus renal ischemia differs from other organs in which C5a is important and the vessels are the main target.

Autoantibodies contribute to IRI in non-renal organs (heart, muscle, intestine), where complement fixation is dependent on autoantibodies to intracellular antigens which are externalized upon ischemia, such as non-muscle myosin heavy chain type II A and C (7, 8). The role of autoantibodies in renal IRI is controversial. In one report, RAG1<sup>-/-</sup> mice were just as susceptible to IRI as wild type (WT) mice (9). In contrast, B cell knockout mice ( $\mu$ MT) were resistant to IRI, which was restored by serum. C3d deposition occurred even in the absence of Ig (10), arguing the classical pathway is not involved and consistent with a role for MBL. Indeed, further studies showed that MBL-deposition occurs in the early reperfusion phase and C3, C6

and C9 later along the tubules and in the peritubular capillaries, with MBL in the same sites. In non-renal IRI, The lectin pathway has also been implicated, as MBL deficient mice are protected from IRI (11). MBL binds to IgM autoantibodies, independent of C1q, which is not required for IRI.

Deficiency of cell surface complement regulators exacerbates renal IRI. Mice genetically deficient in CD59, the major regulator of the membrane attack complex (C5b-9), were more susceptible to IRI and had more C9 deposition along the tubules than WT mice (12), although another CD59 deficient strain were not (13). Deficiency of CD55 (Decay Accelerating Factor) promoted renal IRI, and this deficiency was synergistic with CD59 deficiency (13). Partial deficiency of Crry (*CR1-related gene/protein y*) increases susceptibility to IRI and increases C3 deposition along the TBM (14).

In biopsies of ATN in humans, C3d, but not C4d, is increased along the TBM (15). Human proximal tubular cells cultured with normal human serum activate the alternative pathway and fix C3 on their surface, along with properdin, terminal complement components and C5b-9, but not C1q or C4 (16). This is probably the reason that C3, C5, C6, C7, C9 and C5b-9 neoantigen are deposited in the TBM in tubulo-interstitial disease (17). In ischemic allografts in humans, MBL deposition occurs early in peritubular capillaries and tubular epithelial cells, similar to the mouse (18). Thus there is evidence that both the alternative and the MBL pathways accounts for the focal C3 along the TBM.

Therapies for ATN based on these observations have had some success in mice, including anti-C5 (19), membranophilic myristoylated CR1(20), C5aR antagonist (21), anti-factor B (22), and inhibition of local C3 synthesis with perfusion with C3 siRNA (23). Both anti-factor B and C3 siRNA decreased C3b accumulation in tubules, as did, curiously, anti-C5 (19, 22, 23). Clinical trials have started with myristoylated CR1, which at last report showed that treatment of donor organs was both feasible and safe (2). Monoclonal anti-C5 has had some success in severe cardiac ischemia (24), and but has not been tested in transplantation. Infusion of myristoylated CR1(20) not only ameliorates IRI in mice, it also inhibits acute cellular rejection (cellular infiltrate, T cell proliferative response and renal function), arguing that early complement activation from ischemia promotes the immune response. This is quite compatible with the observations in humans that DGF increases the risk of acute rejection (4).

## **II. T cell mediated rejection**

A characteristic feature of acute cellular rejection in humans (which persists in later biopsies) is abundant C3 deposition along the TBM in a segmental linear pattern, which exceeds the "normal" pattern (4). C3 is often pronounced in atrophic tubules. C5b-9 is also deposited along the TBM in about 30% of cases (25). Most of the C3 derives from local synthesis, which increases in rejection (26, 27). Allografts contribute about 4% of the circulating C3, increasing to 10% during rejection (28). Peritubular C3 is largely derived from proximal tubular cells, as judged by C3 allotype antibodies; donor specific C3 mRNA can be detected in rejecting renal allografts by PCR (29). Tubular synthesis of C3 in vitro is promoted by exposure to IFN $\gamma$  (30), IL-17 (31) or IL-2 (32) and in biopsies correlates with local IFN $\gamma$  production (27). Synthesis of

other complement components by the kidney has been demonstrated, such as C2, C4, and factor B (see ref (32)). Furthermore other cell types such as endothelial cells can make C3.

In mice intragraft synthesis of both C3 and C4 also rises substantially during acute rejection (33). That the intragraft C3 synthesis might be pathogenetically important was established in a seminal publication in mice (30). In these studies congenic C3 gene-disrupted C57BL/6<sup>H-2b</sup> (C3<sup>-/-</sup>) or normal B6 kidneys were transplanted into normal B10.Br<sup>H-2k</sup> recipients. Median graft survival was increased from 12.5 days with WT kidneys to >100 days in the C3<sup>-/-</sup> allografts. The C3 status of the recipient was irrelevant; prolonged survival occurred only when the allograft was C3 deficient. C3 and C5b-9 were deposited along the TBM in WT but not C3<sup>-/-</sup>-kidneys. Rejection appeared to be T cell-mediated, since the lesions were tubulitis and endothelialitis. Acute rejection was not affected by blocking the classical pathway by local or systemic deficiency in C4 (33), suggesting that the mechanism was not via antibody mediated rejection.

Recipients of C3<sup>-/-</sup> kidneys have a reduced alloantigen driven proliferative response of T cells, leading to the conclusion that C3 in graft cells promotes antigen presentation to T cells. Human T cells express CR1 and CR2 and are able to bind to C3b and C3d. In mice the combined gene product (CR1/2) is restricted to <5% of CD4+ cells, which expands during rejection (30). Dendritic cells synthesize C3 which is required for normal T cell priming (34). Conversely, absence of a complement regulator DAF from dendritic cells increases the immune response (35). The complex molecular mechanisms appear to involve alterations in IL-12 production, IFN $\gamma$ , C5a, and Foxp3 cells, but the mechanisms are not yet established (35).

That local C3 synthesis in the graft is also relevant to the human has been suggested by the unexpected finding that the C3 allotype of the graft, but not the donor influences outcome (36). C3 has two main allotypes, F (fast) and S (slow) in the human, caused by a single nucleotide substitution that leads to a change from glycine (C3F) to arginine (C3S) at position 80. Among 513 recipients, graft survival was significantly lower with a C3S/S donor allotype than with C3F/F or C3F/S donor allotypes (hazard ratio 2.2). Graft function was also significantly worse (P<0.001). The effect was restricted to recipients who did not themselves possess the F allele, suggesting that the benefit of the F allotype can be achieved with C3 from the recipient. The C3F/S mutation is on the surface of the molecule and may affect interactions with receptors; alternatively the beneficial effect is due to a linkage with other unidentified genes. The C3F allotype has been linked to other immunologically mediated renal disease such IgA nephropathy and MPGN (see ref (36))

### **III. Antibody Mediated Rejection**

Complement is of course involved in antibody mediated graft injury, as Bordet would have predicted. Demonstration of C4d in peritubular capillaries remains the most robust indicator of circulating anti-donor HLA or ABO antibodies a topic that has been extensively and recently reviewed (1). In the last decade, four forms of antibody mediated graft injury have been defined (4, 37-39): hyperacute, acute and chronic rejection and accommodation. Thus diagnosis of these conditions depends on the combination of C4d deposition and specific histologic or ultrastructural lesions.

Antibodies to donor HLA class I or II antigens are present in 88-95% of the patients who have C4d deposition and acute graft dysfunction vs. less than 10% in C4d negative acute rejection (40-42). Antibodies to donor ABO antigens show a similar association. C4d deposition without detectable circulating antibody can be due to absorption by the graft, as demonstrated by elution of anti-HLA antibodies from rejected grafts in patients who had no detectable circulating antibody at the time, even from needle biopsies (43). Non-HLA, non-ABO antigens are the target in a minority of cases, probably accounting for the rare C4d+ acute rejection in HLA-identical grafts (<2% of patients) (44).

Whether complement fixation is necessary for the pathogenesis of antibody mediated rejection is of some interest. Complement fixation is strongly associated with the ability of antibody to mediate AHR in animal models (45). Antibodies of different isotypes vary in their ability to fix complement. In humans, IgG1,2,3 and IgM fix complement by the classical pathway; IgG4 and IgA do not). Anti-donor antibodies of the strong complement fixing subclass, IgG3, were present in patients with acute rejection, but not in stable patients, whereas the latter had a significant rise only in the non-complement fixing, IgG4 subclass (46).

Wahrmann and colleagues in Vienna first described a method to measure complement fixing antibodies by testing FlowPRA beads coated with HLA antigens for their ability to stain for C4d by flow cytometry after incubation in plasma (47, 48). C1q and C3d/b could also be detected, but the C3 was taken up independent of DSA. This test has been used to determine the clinical significance of complement fixing DSA. Recipients with anti-donor HLA class I antibodies that fix C4d to FlowPRA beads had inferior graft survival compared with those that did not, the latter having a similar outcome to patients with no anti-donor antibody (48). Complement dependent cytotoxicity (CDC-PRA) was also predictive of poorer outcome. Of note, complement-fixing HLA class II antibodies did not affect graft survival, even though associated with C4d deposition in the graft. These studies were extended to cardiac grafts by Smith and colleagues who tested pre-transplant sera on Luminex beads coated with HLA antigens for their C4d fixing ability (49). They found that DSA that fixed complement was associated with a decreased graft survival compared to those with non-complement fixing DSA (graft survival at one year decreasing from 54% to 20%).

**Other complement components** (1). C3 is the next component in the classic pathway sequence after C4, and therefore its cleavage products should indicate more complete complement activation. C3d (or C3c) was found in PTC in 39-60% of biopsies from HLA mismatched grafts with diffuse PTC C4d (42, 50-52). In general C3d and C4d were correlated. However, in one report, 19% of those with C3d had no C4d (51). This finding may be related to C3 activation via the alternative pathway, independent of C4. In the most comprehensive study, C3d was found only in conjunction with C4d in sensitized patients (42). C3d deposition correlated with AHR in all studies of ABO compatible grafts (42, 50-52). Neutrophils in PTC or features of thrombotic microangiopathy correlated with C3d deposition in one study (42) but not two others (42, 52). The pathologic features of C3d+C4d+ biopsies were similar to those with C3d-C4d+ cases in ABO compatible grafts (42). The presence of C3d was associated with increased risk of graft loss, compared to C3d negative cases, but C3d provided no convincing additional risk compared with C4d+. Macrophages in glomeruli correlated with C3c and C4d in glomeruli, which had a worse prognosis than C4d alone (53). The interpretation of C3d is complicated by the common

presence of C3d along the TBM (42). C3d added little diagnostic value to C4d in positive crossmatch grafts showing histologic features of AHR (42). Similarly, Herman et al found that, in contrast to C4d, C3d was not associated with neutrophils in PTC, donor reactive antibodies or outcome (52). Thus at this time no strong argument can be made for including C3d except in the panel for ABO incompatible grafts.

Other complement components, such as C1q and C5b-9 (membrane attack complex; MAC) are not conspicuous in PTC in acute rejection. MAC deposits in tubular basement membranes, rather than PTC (54), perhaps because of the expression of the inhibitor of MAC formation, CD59 in PTC. Lectin pathway components, which activate C4 by binding to microbial carbohydrates, are sometimes detected in conjunction with C4d. Mannose binding lectin-associated serine protease-1 (MASP-1) was present in 1/11 protocol biopsies with C4d; no MBL was detected (50). Among 18 biopsies with C4d, 16 had diffuse H-ficolin along the PTC, whereas none of the 42 cases without C4d had H-ficolin. No MASP-1 or MASP-2 was detectable (55). The significance of these observations is not clear, since MASP proteins are required to activate C4 via the ficolins or MBL. C-reactive protein (CRP) can also activate C4 but generally does not lead to full complement pathway activation (37).

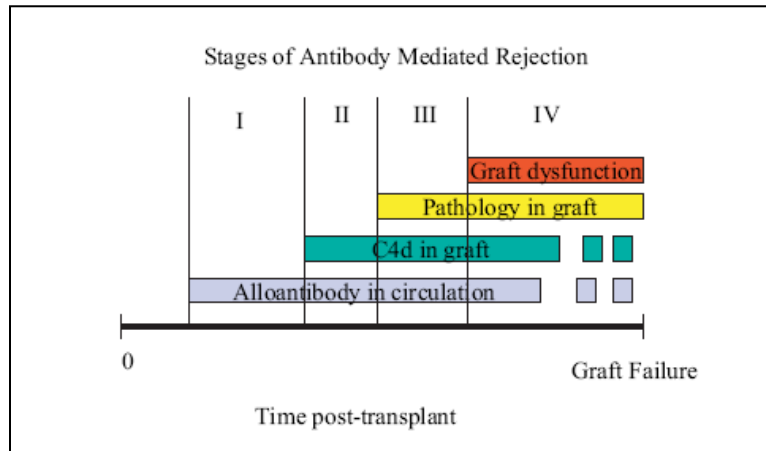
In addition to acute humoral rejection, over the last 5 years, evidence has accumulated that argues for a significant role of alloantibodies to MHC antigens in the pathogenesis of slowly progressive graft injury and dysfunction (56), a process now defined in the Banff system as chronic, active antibody mediated rejection (57). The components are listed below:

***Diagnostic Criteria for Chronic Antibody Mediated Rejection (CHR) (4, 58)***

1. Histologic evidence of chronic injury: need 2 of 4
  - Arterial intimal fibrosis without elastosis
  - Duplication of glomerular basement membrane
  - Multi-laminated PTC basement membrane
  - Interstitial fibrosis with tubular atrophy
2. Evidence for Ab action/deposition in tissue (e.g., C4d in PTC)
3. Serologic evidence of anti-HLA or other antidonor antibody

The most tightly associated pathological feature is transplant glomerulopathy (duplication of the GBM) (59), although some have also found an association with chronic transplant arteriopathy (56), or even just interstitial fibrosis (60). C4d is deposited in glomerular capillaries (as best shown in paraffin sections) and well as in PTC, which may be sparse. Overall in transplant glomerulopathy, about 30% will have C4d, and about 70% will have DSA, most often with class II specificity (61). The C4d, DSA negative cases may be a late stage of CHR or due to non-antibody mediated injury (TMA from calcineurin inhibitor toxicity or T cell mediated glomerular damage). CHR progresses through 4 stages as shown in non-human primates (62) and in rare clinical cases (unpublished observations) (1). Murine studies have demonstrated that complement fixing antibodies to graft class I MHC antigens are sufficient to induce chronic transplant arteriopathy in RAG-1<sup>-/-</sup> mice (63).

Reprinted  
from (1)



**Complement effects on the endothelium (38).** The primary target of antibody mediated rejection is in the capillary and arterial endothelium. Complement fixation on the endothelium can lead to cytolysis, via C5b-9 (MAC) (64) and complement can activate the endothelial cells, the latter relevant to both acute and chronic rejection. Exposure to MAC in sublytic concentrations, as soluble C5b-9, or C3a and C5a, increases expression of E-selectin, ICAM-1, and VCAM-1 on cultured endothelial cells (65). MAC elicits signals for endothelial-cell proliferation, as shown by the release of PDGF and bFGF (66), and for the production of the chemokines CCL2, IL-8 and CCL5, through stimulation of IL-1 $\alpha$  production (67). C3a and C5a increase the endothelial expression of cytokines and chemokines (such as IL-6, IL-8, IL-1 $\alpha$  and CCL5), and promote signaling through the MAP kinase pathway (68, 69). Antibodies also have effects on the endothelium independent of antibody. In vitro anti-MHC class I antibodies promote endothelial proliferation via increased expression of basic FGF receptors, increased phosphorylation of Src, and NF- $\kappa$ B levels (70). In mice, non-complement fixing IgG alloantibodies are associated with graft acceptance, but can also activate endothelial cells to produce chemokines and promote rejection (45). Further studies will be needed to determine whether complement fixation is necessary for chronic rejection. Recent studies in the mouse indicate that the chronic arteriopathy can be induced in C3 deficient RAG1 $^{-/-}$  mice give passive antibody (Hirohashi et al unpublished).

**Complement effects on B cells(38).** The magnitude of the IgG response to alloantigens are dependent on C3 and C4, but not C5, as shown using skin grafts in C3, C4 or C5 knockout mice (71). B cells and dendritic cells express the complement receptors CD35 (CR1, C3b -C4b) and CD21 (C3d receptor), and are therefore able to retain antigen covalently linked to C3 or C4. Engagement of CD21 lowers the threshold for B-cell activation and thereby acts as a natural adjuvant (72). This also promotes B cell antigen presentation to T cells for those antigens also able to bind to that B cell Ig (73). The prominent accumulation of C4d and C3d in normal germinal centers can be taken as evidence for complement participation in B cell activation (74).

**Practical aspects of C4d staining.** The Banff03 defined positive C4d by immunofluorescence as "widespread, strong linear circumferential peritubular capillary (PTC) staining in cortex or medulla", often interpreted as >50% of the capillaries. Authors have used a variety of cutoffs for

the <50% cases. Banff 2007 has added more specific definitions of C4d extent, with the intention that comparisons will be easier between publications (Solez et al, submitted).

#### Banff 2007 C4d Scores (% of biopsy with PTC+)

C4d0: Negative:	0%
C4d1: Minimal C4d stain/detection:	<10%
C4d2: Focal C4d stain/positive:	10-50%
C4d3: Diffuse C4d stain/positive:	>50%

As stated in the recent Banff meeting report (Solez et al, submitted), the interpretation of C4d staining should be adjusted for the applied technique. IHC on paraffin section is usually less sensitive by about one grade level (i.e. diffuse staining on IF (cryosections) can be seen as focal on IHC (paraffin sections) (75, 76). Therefore, the report should indicate the actual % of tissue involved and the potential clinical significance. For example, diffuse positive C4d by IF or IHC is highly correlated with circulating anti-donor antibody. Focal positive C4d by IHC is possibly equivalent to diffuse positive IF, and should be re-tested on IF, if possible. The clinical significance of focal positive C4d by IF or minimal C4d by IHC is unknown. Meehan and colleagues found that focal C4d in PTC had one year graft losses no different from C4d negative cases (77). In contrast, Magil and Tinckam found that the outcome of focal C4d was worse than those without C4d (78). C4d staining of  $\geq 25\%$  of the PTCs by immunohistochemistry was associated with decreased one year graft survival (79).

#### IV. Accommodation

Accommodation is defined as normal graft function (and histology) in the presence of antibodies to donor antigens (80). This has been observed most commonly in transplants across an ABO barrier after pre-transplant reduction of antibodies. Remarkably, even if the ABO antibodies return after 4-6 weeks, no obvious graft rejection occurs. Stable ABO incompatible grafts show differences in signaling pathways and cytokines by microarray gene expression analysis and notably increased levels of muc-1 in glomerular capillaries (80). Accommodation in ABO incompatible grafts is not due to a change in the nature of the antibody or to loss of the target antigen, since C4d is deposited in the renal microcirculation. Protocol biopsies have revealed C4d along the PTC in 25-80% of ABO incompatible grafts, with evidence of AHR in only 4-12% (42, 81). Either the full complement pathway is not activated, or the endothelium develops resistance to its effects. A large study was designed to test whether C3d could distinguish those with accommodation, from those with acute humoral rejection (42). About 40% of the C4d+ biopsies (14/37) had C3d, and C3d correlated somewhat with histologic features of acute humoral rejection, however 70% of the C3d+ biopsies also had no evidence of injury, arguing that accommodation occurs distal to C3 activation (perhaps via inhibition of MAC). In any case, C4d deposition in ABO incompatible grafts and therefore is of limited diagnostic value.

C4d deposition also occurs in 2-26% of histologically normal ABO compatible grafts, the higher frequency found in HLA-presensitized patients (42, 82). In these patients incidental C4d deposition does not necessary portend acute humoral rejection, however, it may not be entirely benign. Among 17 patients who had C4d in PTC without histologic evidence of acute humoral

or cellular rejection, and who received no increased immunosuppression, graft loss at 3 years was 32% compared with 0% among those 5 patients treated with increased immunosuppression, suggesting that incidental C4d may represent a “smoldering’ rejection” (83).

The most recent Banff meeting (2007) agreed to include a new diagnostic category, “ C4d deposition without morphologic evidence of active rejection” (Solez et al, submitted). The criteria are:

- C4d+ peritubular capillaries
- Circulating anti-donor antibodies
- No histologic sign of active rejection, including g0, cg0, ptc0 and no ptc lamination

Cases with simultaneous borderline changes or acute tubular necrosis are considered as indeterminate. The term accommodation was not used, because the long term stability of this condition has not been established.

Accommodation may have different degrees of effectiveness and stability, ranging from none (hyperacute rejection), to minimal (acute rejection), substantial (chronic rejection), or complete (stable accommodation). The minimal features that indicate transformation from accommodation to rejection have yet to be defined. Controlled trials and further follow-up will be needed to interpret the long-term significance of “incidental” C4d deposition. Until convincing evidence is reported, the default position is that development of donor reactive HLA antibodies and/or C4d deposition should trigger a heightened state of clinical vigilance (1).

## REFERENCES

1. Colvin RB, Antibody-mediated renal allograft rejection: diagnosis and pathogenesis. *J Am Soc Nephrol*, 2007; 18: 1046-56.
2. Zhou W, Medof ME, Heeger PS, Sacks S, Graft-derived complement as a mediator of transplant injury. *Curr Opin Immunol*, 2007; 19: 569-76.
3. Brook NR, White SA, Waller JR, Veitch PS, Nicholson ML, Non-heart beating donor kidneys with delayed graft function have superior graft survival compared with conventional heart-beating donor kidneys that develop delayed graft function. *Am J Transplant*, 2003; 3: 614-8.
4. Colvin RB, Nickleleit V, *Renal transplant pathology*, in *Heptinstall's Pathology of the Kidney*, J.C. Jennette, et al., Editors. 2006, Lippincott-Raven: Philadelphia. p. 1347-490.
5. Zhou W, Farrar CA, Abe K, Pratt JR, Marsh JE, Wang Y, Stahl GL, Sacks SH, Predominant role for C5b-9 in renal ischemia/reperfusion injury. *J Clin Invest*, 2000; 105: 1363-71.
6. Selander B, Martensson U, Weintraub A, Holmstrom E, Matsushita M, Thiel S, Jensenius JC, Truedsson L, Sjöholm AG, Mannan-binding lectin activates C3 and the alternative complement pathway without involvement of C2. *J Clin Invest*, 2006; 116: 1425-34.
7. Austen WG, Jr., Zhang M, Chan R, Friend D, Hechtman HB, Carroll MC, Moore FD, Jr., Murine hindlimb reperfusion injury can be initiated by a self-reactive monoclonal IgM. *Surgery*, 2004; 136: 401-6.
8. Zhang M, Alicot EM, Chiu I, Li J, Verna N, Vorup-Jensen T, Kessler B, Shimaoka M, Chan R, Friend D, Mahmood U, Weissleder R, Moore FD, Carroll MC, Identification of the target self-antigens in reperfusion injury. *J Exp Med*, 2006; 203: 141-52.
9. Park P, Haas M, Cunningham PN, Bao L, Alexander JJ, Quigg RJ, Injury in renal ischemia-reperfusion is independent from immunoglobulins and T lymphocytes. *Am J Physiol Renal Physiol*, 2002; 282: F352-7.
10. Burne-Taney MJ, Ascon DB, Daniels F, Racusen L, Baldwin W, Rabb H, B cell deficiency confers protection from renal ischemia reperfusion injury. *J Immunol*, 2003; 171: 3210-5.
11. Zhang M, Takahashi K, Alicot EM, Vorup-Jensen T, Kessler B, Thiel S, Jensenius JC, Ezekowitz RA, Moore FD, Carroll MC, Activation of the lectin pathway by natural IgM in a model of ischemia/reperfusion injury. *J Immunol*, 2006; 177: 4727-34.
12. Turnberg D, Botto M, Lewis M, Zhou W, Sacks SH, Morgan BP, Walport MJ, Cook HT, CD59a deficiency exacerbates ischemia-reperfusion injury in mice. *Am J Pathol*, 2004; 165: 825-32.
13. Yamada K, Miwa T, Liu J, Nangaku M, Song WC, Critical protection from renal ischemia reperfusion injury by CD55 and CD59. *J Immunol*, 2004; 172: 3869-75.
14. Thurman JM, Ljubanovic D, Royer PA, Kraus DM, Molina H, Barry NP, Proctor G, Levi M, Holers VM, Altered renal tubular expression of the complement inhibitor Crry permits complement activation after ischemia/reperfusion. *J Clin Invest*, 2006; 116: 357-68.
15. Thurman JM, Lucia MS, Ljubanovic D, Holers VM, Acute tubular necrosis is characterized by activation of the alternative pathway of complement. *Kidney Int*, 2005; 67: 524-30.
16. Biancone L, David S, Della Pietra V, Montrucchio G, Cambi V, Camussi G, Alternative pathway activation of complement by cultured human proximal tubular epithelial cells. *Kidney Int*, 1994; 45: 451-60.
17. Khan TN, Sinniah R, Role of complement in renal tubular damage. *Histopathology*, 1995; 26: 351-6.
18. de Vries B, Walter SJ, Peutz-Kootstra CJ, Wolfs TG, van Heurn LW, Buurman WA, The mannose-binding lectin-pathway is involved in complement activation in the course of renal ischemia-reperfusion injury. *Am J Pathol*, 2004; 165: 1677-88.
19. De Vries B, Matthijsen RA, Wolfs TG, Van Bijnen AA, Heeringa P, Buurman WA, Inhibition of complement factor C5 protects against renal ischemia-reperfusion injury: inhibition of late apoptosis and inflammation. *Transplantation*, 2003; 75: 375-82.
20. Pratt JR, Jones ME, Dong J, Zhou W, Chowdhury P, Smith RA, Sacks SH, Nontransgenic hyperexpression of a complement regulator in donor kidney modulates transplant ischemia/reperfusion damage, acute rejection, and chronic nephropathy. *Am J Pathol*, 2003; 163: 1457-65.
21. Arumugam TV, Shiels IA, Strachan AJ, Abbenante G, Fairlie DP, Taylor SM, A small molecule C5a receptor antagonist protects kidneys from ischemia/reperfusion injury in rats. *Kidney Int*, 2003; 63: 134-42.
22. Thurman JM, Royer PA, Ljubanovic D, Dursun B, Lenderink AM, Edelstein CL, Holers VM, Treatment with an inhibitory monoclonal antibody to mouse factor B protects mice from induction of apoptosis and renal ischemia/reperfusion injury. *J Am Soc Nephrol*, 2006; 17: 707-15.

23. Zheng X, Feng B, Chen G, Zhang X, Li M, Sun H, Liu W, Vladau C, Liu R, Jevnikar AM, Garcia B, Zhong R, Min WP, Preventing renal ischemia-reperfusion injury using small interfering RNA by targeting complement 3 gene. *Am J Transplant*, 2006; 6: 2099-108.
24. Smith PK, Carrier M, Chen JC, Haverich A, Levy JH, Menasche P, Shernan SK, Van de Werf F, Adams PX, Todaro TG, Verrier E, Effect of pexelizumab in coronary artery bypass graft surgery with extended aortic cross-clamp time. *Ann Thorac Surg*, 2006; 82: 781-8; discussion 88-9.
25. Endo T, Nakao S, Koizumi K, Nishio M, Fujimoto K, Sakai T, Kumano K, Obara M, Koike T, Successful treatment with rituximab for autoimmune hemolytic anemia concomitant with proliferation of Epstein-Barr virus and monoclonal gammopathy in a post-nonmyeloablative stem cell transplant patient. *Ann Hematol*, 2004; 83: 114-6.
26. Andrews PA, Pani A, Zhou W, Sacks SH, Local transcription of complement C3 in human allograft rejection. Evidence for a pathogenic role and correlation to histology and outcome. *Transplantation*, 1994; 58: 637-40.
27. Serinsoz E, Bock O, Gwinner W, Schwarz A, Haller H, Kreipe H, Mengel M, Local complement C3 expression is upregulated in humoral and cellular rejection of renal allografts. *Am J Transplant*, 2005; 5: 1490-4.
28. Tang S, Zhou W, Sheerin NS, Vaughan RW, Sacks SH, Contribution of renal secreted complement C3 to the circulating pool in humans. *J Immunol*, 1999; 162: 4336-41.
29. Andrews PA, Finn JE, Lloyd CM, Zhou W, Mathieson PW, Sacks SH, Expression and tissue localization of donor-specific complement C3 synthesized in human renal allografts. *European Journal of Immunology*, 1995; 25: 1087-93.
30. Pratt JR, Basheer SA, Sacks SH, Local synthesis of complement component C3 regulates acute renal transplant rejection. *Nat Med*, 2002; 8: 582-7.
31. Van Kooten C, Boonstra JG, Paape ME, Fossiez F, Banchereau J, Lebecque S, Bruijn JA, De Fijter JW, Van Es LA, Daha MR, Interleukin-17 activates human renal epithelial cells in vitro and is expressed during renal allograft rejection. *Journal of the American Society of Nephrology*, 1998; 9: 1526-34.
32. Brooimans RA, Stegmann AP, van Dorp WT, van der Ark AA, van der Woude FJ, van Es LA, Daha MR, Interleukin 2 mediates stimulation of complement C3 biosynthesis in human proximal tubular epithelial cells. *J Clin Invest*, 1991; 88: 379-84.
33. Lin T, Zhou W, Farrar CA, Hargreaves RE, Sheerin NS, Sacks SH, Deficiency of C4 from donor or recipient mouse fails to prevent renal allograft rejection. *Am J Pathol*, 2006; 168: 1241-8.
34. Peng Q, Li K, Patel H, Sacks SH, Zhou W, Dendritic cell synthesis of C3 is required for full T cell activation and development of a Th1 phenotype. *J Immunol*, 2006; 176: 3330-41.
35. Lalli PN, Strainic MG, Lin F, Medof ME, Heeger PS, Decay accelerating factor can control T cell differentiation into IFN-gamma-producing effector cells via regulating local C5a-induced IL-12 production. *J Immunol*, 2007; 179: 5793-802.
36. Brown KM, Kondeatis E, Vaughan RW, Kon SP, Farmer CK, Taylor JD, He X, Johnston A, Horsfield C, Janssen BJ, Gros P, Zhou W, Sacks SH, Sheerin NS, Influence of donor C3 allotype on late renal-transplantation outcome. *N Engl J Med*, 2006; 354: 2014-23.
37. Rotman S, Collins AB, Colvin RB, C4d deposition in allografts: Current concepts and interpretation. *Transplantation Reviews*, 2005; 19: 65-77.
38. Colvin RB, Smith RN, Antibody-mediated organ-allograft rejection. *Nat Rev Immunol*, 2005; 5: 807-17.
39. Takemoto SK, Zeevi A, Feng S, Colvin RB, Jordan S, Kobashigawa J, Kupiec-Weglinski J, Matas A, Montgomery RA, Nickerson P, Platt JL, Rabb H, Thistlethwaite R, Tyan D, Delmonico FL, National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J Transplant*, 2004; 4: 1033-41.
40. Mauyyedi S, Crespo M, Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Tolkoff-Rubin NE, Williams WW, Delmonico FL, Cosimi AB, Colvin RB, Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J Am Soc Nephrol*, 2002; 13: 779-87.
41. Bohmig GA, Exner M, Habicht A, Schillinger M, Lang U, Kletzmayer J, Saemann MD, Horl WH, Watschinger B, Regele H, Capillary C4d deposition in kidney allografts: a specific marker of alloantibody-dependent graft injury. *J Am Soc Nephrol*, 2002; 13: 1091-9.
42. Haas M, Rahman MH, Racusen LC, Kraus ES, Bagnasco SM, Segev DL, Simpkins CE, Warren DS, King KE, Zachary AA, Montgomery RA, C4d and C3d staining in biopsies of ABO- and HLA-incompatible renal allografts: correlation with histologic findings. *Am J Transplant*, 2006; 6: 1829-40.

43. Martin L, Guignier F, Bocrie O, D'Athis P, Rageot D, Rifle G, Justrabo E, Mousson C, Detection of anti-HLA antibodies with flow cytometry in needle core biopsies of renal transplants recipients with chronic allograft nephropathy. *Transplantation*, 2005; 79: 1459-61.
44. Collins AB, Chicano S, Cornell LD, Tolkoff-Rubin N, Goes NB, Farrell ML, Cosimi AB, Colvin RB, Putative antibody-mediated rejection with C4d deposition in HLA-identical, ABO compatible renal allografts. *Transplantation Proc*, 2006; in press.
45. Rahimi S, Qian Z, Layton J, Fox-Talbot K, Baldwin WM, 3rd, Wasowska BA, Non-complement- and complement-activating antibodies synergize to cause rejection of cardiac allografts. *Am J Transplant*, 2004; 4: 326-34.
46. Gao ZH, McAlister VC, Wright JR, Jr., McAlister CC, Peltekian K, MacDonald AS, Immunoglobulin-G subclass antidonor reactivity in transplant recipients. *Liver Transpl*, 2004; 10: 1055-9.
47. Wahrmann M, Exner M, Regele H, Derfler K, Kormoczi GF, Lhotta K, Zlabinger GJ, Bohmig GA, Flow cytometry based detection of HLA alloantibody mediated classical complement activation. *J Immunol Methods*, 2003; 275: 149-60.
48. Wahrmann M, Exner M, Schillinger M, Haidbauer B, Regele H, Kormoczi GF, Horl WH, Bohmig GA, Pivotal role of complement-fixing HLA alloantibodies in presensitized kidney allograft recipients. *Am J Transplant*, 2006; 6: 1033-41.
49. Smith JD, Hamour IM, Banner NR, Rose ML, C4d fixing, luminex binding antibodies - a new tool for prediction of graft failure after heart transplantation. *Am J Transplant*, 2007; 7: 2809-15.
50. Sund S, Hovig T, Reisaeter AV, Scott H, Bentdal O, Mollnes TE, Complement activation in early protocol kidney graft biopsies after living-donor transplantation. *Transplantation*, 2003; 75: 1204-13.
51. Kuypers DR, Lerut E, Evenepoel P, Maes B, Vanrenterghem Y, Van Damme B, C3D deposition in peritubular capillaries indicates a variant of acute renal allograft rejection characterized by a worse clinical outcome. *Transplantation*, 2003; 76: 102-8.
52. Herman J, Lerut E, Van Damme-Lombaerts R, Emonds MP, Van Damme B, Capillary deposition of complement C4d and C3d in pediatric renal allograft biopsies. *Transplantation*, 2005; 79: 1435-40.
53. Molne J, Nyberg G, Blohme I, Rydberg L, Breimer ME, Svalander CT, Glomerular C3c deposition and intravascular macrophage accumulation in early humoral renal allograft rejection signifies a poor short-term outcome. *Apmis*, 2006; 114: 700-11.
54. Nishi S, Imai N, Ito Y, Ueno M, Fukase S, Mori H, Arakawa M, Bassam A, Saito K, Takahashi K, Gejyo F, Pathological study on the relationship between C4d, CD59 and C5b-9 in acute renal allograft rejection. *Clin Transplant*, 2004; 18 Suppl 11: 18-23.
55. Imai N, Nishi S, Alchi B, Ueno M, Fukase S, Arakawa M, Saito K, Takahashi K, Gejyo F, Immunohistochemical evidence of activated lectin pathway in kidney allografts with peritubular capillary C4d deposition. *Nephrol Dial Transplant*, 2006; 21: 2589-95.
56. Mauiyyedi S, Pelle PD, Saidman S, Collins AB, Pascual M, Tolkoff-Rubin NE, Williams WW, Cosimi AA, Schneeberger EE, Colvin RB, Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *Journal of the American Society of Nephrology*, 2001; 12: 574-82.
57. Solez K, Colvin RB, Racusen LC, Sis B, Halloran PF, Birk PE, Campbell PM, Cascalho M, Collins AB, Demetris AJ, Drachenberg CB, Gibson IW, Grimm PC, Haas M, Lerut E, Liapis H, Mannon RB, Marcus PB, Mengel M, Mihatsch MJ, Nankivell BJ, Nicleleit V, Papadimitriou JC, Platt JL, Randhawa P, Roberts I, Salinas-Madruga L, Salomon DR, Seron D, Sheaff M, Weening JJ, Banff '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant*, 2007; 7: 518-26.
58. Solez K, Colvin RB, Racusen L, Sis B, Halloran PH, Birk P, Cascalho M, Collins AB, Campbell PM, Demetris A, Banff '05 meeting report: Differential diagnosis of chronic injury and elimination of chronic allograft nephropathy ('CAN') in the Banff schema. *American J. Transplantation*, in press.
59. Regele H, Bohmig GA, Habicht A, Gollowitzer D, Schillinger M, Rockenschaub S, Watschinger B, Kerjaschki D, Exner M, Capillary deposition of complement split product C4d in renal allografts is associated with basement membrane injury in peritubular and glomerular capillaries: a contribution of humoral immunity to chronic allograft rejection. *J Am Soc Nephrol*, 2002; 13: 2371-80.
60. David-Neto E, Prado E, Beutel A, Ventura CG, Siqueira SA, Hung J, Lemos FB, de Souza NA, Nahas WC, Ianhez LE, David DR, C4d-positive chronic rejection: a frequent entity with a poor outcome. *Transplantation*, 2007; 84: 1391-8.

61. Sis B, Campbell PM, Mueller T, Hunter C, Cockfield SM, Cruz J, Meng C, Wishart D, Solez K, Halloran PF, Transplant glomerulopathy, late antibody-mediated rejection and the ABCD tetrad in kidney allograft biopsies for cause. *Am J Transplant*, 2007; 7: 1743-52.
62. Smith RN, Kawai T, Boskovic S, Nadazdin O, Sachs DH, Cosimi AB, Colvin RB, Chronic antibody mediated rejection of renal allografts: pathological, serological and immunologic features in nonhuman primates. *Am J Transplant*, 2006; 6: 1790-8.
63. Uehara S, Chase CM, Cornell LD, Madsen JC, Russell PS, Colvin RB, Chronic cardiac transplant arteriopathy in mice: relationship of alloantibody, C4d deposition and neointimal fibrosis. *Am J Transplant*, 2007; 7: 57-65.
64. Nakashima S, Qian Z, Rahimi S, Wasowska BA, Baldwin WM, 3rd, Membrane attack complex contributes to destruction of vascular integrity in acute lung allograft rejection. *J Immunol*, 2002; 169: 4620-7.
65. Saadi S, Holzknrecht RA, Patte CP, Platt JL, Endothelial cell activation by pore-forming structures: pivotal role for interleukin-1alpha. *Circulation*, 2000; 101: 1867-73.
66. Benzaquen LR, Nicholson-Weller A, Halperin JA, Terminal complement proteins C5b-9 release basic fibroblast growth factor and platelet-derived growth factor from endothelial cells. *Journal of Experimental Medicine*, 1994; 179: 985-92.
67. Selvan RS, Kapadia HB, Platt JL, Complement-induced expression of chemokine genes in endothelium: regulation by IL-1-dependent and -independent mechanisms. *J Immunol*, 1998; 161: 4388-95.
68. Albrecht EA, Chinnaiyan AM, Varambally S, Kumar-Sinha C, Barrette TR, Sarma JV, Ward PA, C5a-induced gene expression in human umbilical vein endothelial cells. *Am J Pathol*, 2004; 164: 849-59.
69. Monsinjon T, Gasque P, Chan P, Ischenko A, Brady JJ, Fontaine MC, Regulation by complement C3a and C5a anaphylatoxins of cytokine production in human umbilical vein endothelial cells. *Faseb J*, 2003; 17: 1003-14.
70. Jin YP, Singh RP, Du ZY, Rajasekaran AK, Rozengurt E, Reed EF, Ligation of HLA class I molecules on endothelial cells induces phosphorylation of Src, paxillin, and focal adhesion kinase in an actin- dependent manner. *J Immunol*, 2002; 168: 5415-23.
71. Marsh JE, Farmer CK, Jurcevic S, Wang Y, Carroll MC, Sacks SH, The allogeneic T and B cell response is strongly dependent on complement components C3 and C4. *Transplantation*, 2001; 72: 1310-8.
72. Dempsey PW, Allison ME, Akkaraju S, Goodnow CC, Fearon DT, C3d of complement as a molecular adjuvant: bridging innate and acquired immunity. *Science*, 1996; 271: 348-50.
73. Prechl J, Baiu DC, Horvath A, Erdei A, Modeling the presentation of C3d-coated antigen by B lymphocytes: enhancement by CR1/2-BCR co-ligation is selective for the co-ligating antigen. *Int Immunol*, 2002; 14: 241-7.
74. Zwirner J, Felber E, Schmidt P, Riethmuller G, Feucht HE, Complement activation in human lymphoid germinal centres. *Immunology*, 1989; 66: 270-7.
75. Nadasdy GM, Bott C, Cowden D, Pelletier R, Ferguson R, Nadasdy T, Comparative study for the detection of peritubular capillary C4d deposition in human renal allografts using different methodologies. *Hum Pathol*, 2005; 36: 1178-85.
76. Seemayer CA, Gaspert A, Nিকেleit V, Mihatsch MJ, C4d staining of renal allograft biopsies: comparative analysis of different staining techniques. *Nephrol Dial Transplant*, 2006.
77. Poduval RD, Kadambi PV, Josephson MA, Cohn RA, Harland RC, Javaid B, Huo D, Manaligod JR, Thistlethwaite JR, Meehan SM, Implications of immunohistochemical detection of C4d along peritubular capillaries in late acute renal allograft rejection. *Transplantation*, 2005; 79: 228-35.
78. Magil AB, Tinckam KJ, Focal peritubular capillary C4d deposition in acute rejection. *Nephrol Dial Transplant*, 2006; 21: 1382-8.
79. Lorenz M, Regele H, Schillinger M, Exner M, Rasoul-Rockenschaub S, Wahrmann M, Kletzmayer J, Silberhumer G, Horl WH, Bohmig GA, Risk factors for capillary C4d deposition in kidney allografts: evaluation of a large study cohort. *Transplantation*, 2004; 78: 447-52.
80. Park WD, Grande JP, Ninova D, Nath KA, Platt JL, Gloor JM, Stegall MD, Accommodation in ABO-incompatible kidney allografts, a novel mechanism of self-protection against antibody-mediated injury. *Am J Transplant*, 2003; 3: 952-60.
81. Fidler ME, Gloor JM, Lager DJ, Larson TS, Griffin MD, Textor SC, Schwab TR, Prieto M, Nyberg SL, Ishitani MB, Grande JP, Kay PA, Stegall MD, Histologic findings of antibody-mediated rejection in ABO blood-group-incompatible living-donor kidney transplantation. *Am J Transplant*, 2004; 4: 101-7.

82. Mengel M, Bogers J, Bosmans J-P, Serón D, Moreso F, Carrera M, Gwinner W, Schwarz A, De Broe M, Kreipel H, Haller H, Incidence of C4d stain in protocol biopsies from renal allografts: Results from a multicenter trial. *Am J Transplant*, 2004; in press.
83. Dickenmann M, Steiger J, Descoeurdes B, Mihatsch M, Nickleit V, The fate of C4d positive kidney allografts lacking histological signs of acute rejection. *Clin Nephrol*, 2006; 65: 173-9.